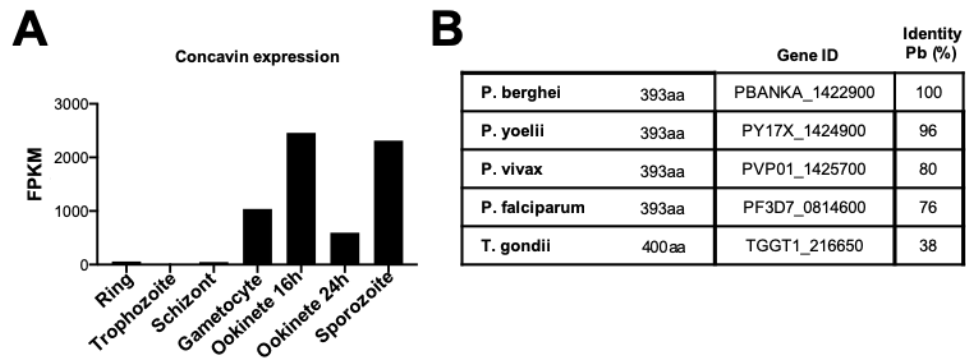


Appendix

Appendix Figure S1	2
Appendix Figure S2	3
Appendix Figure S3	4
Appendix Figure S4	5
Appendix Figure S5	6
Appendix Figure S6	7
Appendix Figure S7	8
Appendix Figure S8	9
Appendix Figure S9	10
Appendix Table S1.....	11

Appendix Figure S1



Appendix Figure S1 | (A) RNAseq abundance of concavin in blood and mosquito stage parasites (B) Sequence identity of *P. berghei* concavin with *P. yoelii*, *P. vivax*, *P. falciparum* and *T. gondii*.

Appendix Figure S2

A

PF3D7_0814600	1	MTNIICTFKTTPDNAKTPDNAIWNQFOYCDEKGWYSLSNHDEITLRPTTFNDKRIKFL
PBANKA_1422900	1	MTNVVCTFKTTPETAKAPDNAIWNRFQYCDEKGWYSLSNHDEITLRPTTFNDGRIKFL
PY17X_1424900	1	MTNVVCTFKAPPETAKAPDNAIWNRFQYCDEKGWYSLSNHDEITLRPTTFNDGRIKFL
PVP01_1425700	1	MTNVVCTFKTTPETAKAPDNAIWNRFQYCDEKGWYSLTNHDEITLRPTTFSDGRIKFL
PF3D7_0814600	61	VQLPEIPSEFESILSGRYDAKAWGKEDCVVIEGDKDVHIRLPGFKEKINYNEERFPTF
PBANKA_1422900	61	POLDTIPEEFESVLCGKYDAKAWGKDDCNLVIEGDKDVHISLPLGKEKINYNEKERFPTF
PY17X_1424900	61	POLDTIPEEFESVLCGKYDAKAWGKDDCNLVIEGDKDVHISLPLGKEKINYNEKERFPTF
PVP01_1425700	61	PQLEKIPEEFESVLCGKYDAKAWGKDDCNLVIEGDKDVHISLPLGQEKINYNEERFPTF
PF3D7_0814600	121	LKNWKIIVSILNEHVTLIRINAEETALIININEKKNVTVKSVDFNNGFLCVNPHTNLAIAIY
PBANKA_1422900	121	LKNSKIIVSLLNENLTVIRINLETALLICINEKKSIVVKSINFNNGFACVNPYSNLAIITY
PY17X_1424900	121	LKNSKIIVSLLNENLTVIRINLETGLLISINEKKSIVVKSINFNNGFACVNPYSNLAIITY
PVP01_1425700	121	LKNWKIIVGMLNEHITVIRINTEETAIIVSINEKSNVTVKCVNFNNGFLCVNPHTNLAIAIY
PF3D7_0814600	181	GDFALSSLLKKCELIQNIPEEGKGWFFTHLFKWGHIIPKELEIKLPSPGLKLIGKKIDT
PBANKA_1422900	181	GGFAFNDLKKCEIVPTITHSCCEWAFFVHLFKWGHIVIPKDLEIKIPSSGLKLIGKKVDT
PY17X_1424900	181	GGFAFNDLKKCEIVPTITHSCCEWAFFVHLFKWGHIVIPKDLEIKIPSSGLKLIGKKVDT
PVP01_1425700	181	GDFALSELKKCELVPNITHECAEWGFFVHLFKWGHIVIPKDIEIKLPSPGLKLIGKKIDT
PF3D7_0814600	241	LAIIVSIPPNIIHVKLIDGPKCIRKLEYGQDYNITAIKSSSEDVVIYILFDGQLLKYEFSF
PBANKA_1422900	241	IAIVSLPPNIIQIHVKIDGPKCIRKVEYGQDYNITAIKSSSEDIDYVLFQGLLKYEFSY
PY17X_1424900	241	IAIVSLPPNIIQIHVKIDGPKCIRKVEYGQDYNITAIKSSSEDIDYVLFQGLLKYEFSY
PVP01_1425700	241	VAIVSLPPNIIYHVKLIDGPKCIRKLEYGQDYSITAIKSSSEDIDYVLFQGLLKYEFSF
PF3D7_0814600	301	DIRLNKPEKGRSLHSAKLKCIKSKSVTSFIFQETKNCKILLGSCNCPDNLGHMLNQTII
PBANKA_1422900	301	DTRLNKEGKGKSIHNAKLKCTSKSKEVSTFIFQESPNCKVLLGSCNCPDNLGHMLNQTII
PY17X_1424900	301	DTRLNKEGKGKSIHNAKLKCTSKSKEVSTFVFOESQNCCKVLLGSCNCPDNLGHMLNQTII
PVP01_1425700	301	DTRLNKVVGKGRSINYAKLKCIKSKSVTSFVFQATANSKLLLDNCNCPDNLGHMLNQTII
PF3D7_0814600	361	AIFDAEIGEYLSHPQGLQLTSVFNTLSYPLDKE
PBANKA_1422900	361	SIFDAEIGEYQSHPOGLLLTEAFEKLSYPVENA
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PVP01_1425700	361	SVFDAETGEYLSHPQGLQLTEVFNTLSYPEKE

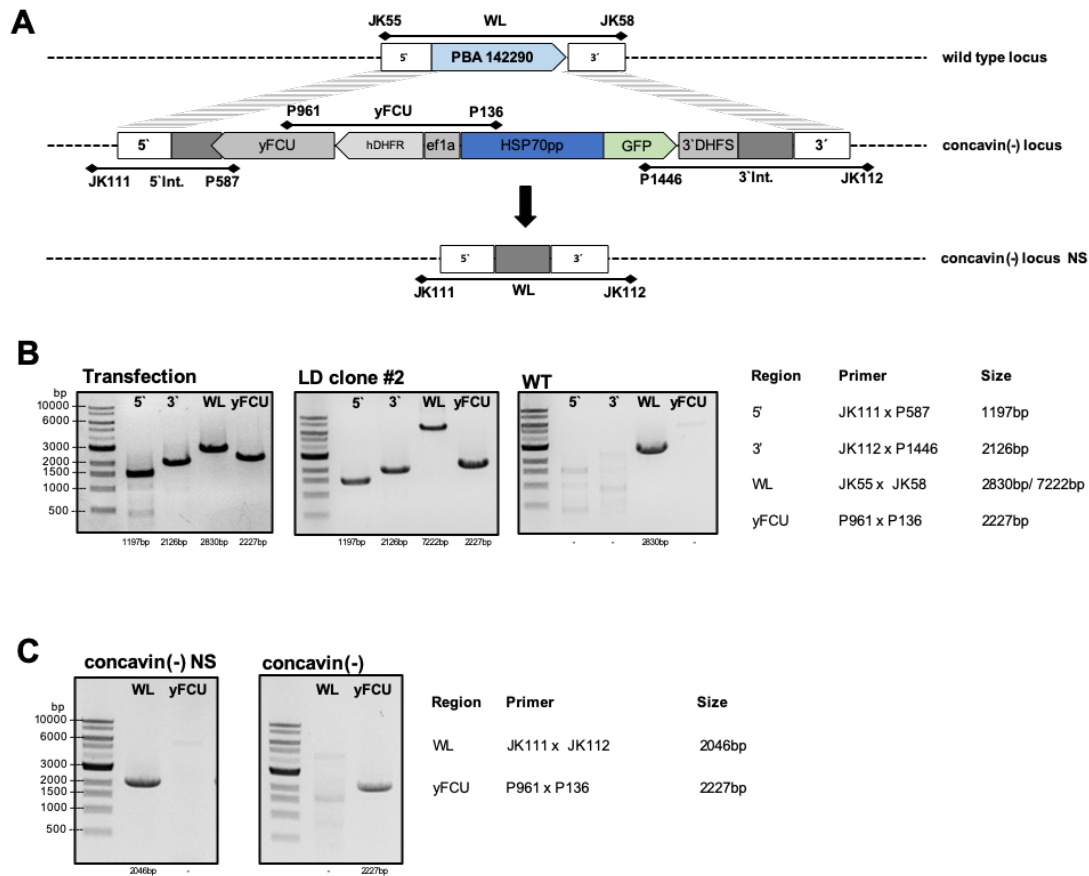
B

TGGT1_216650	1	MERQATCRYDPL-VEVPLPGIIVWTOHQYVDGAGWLALPDREKLELKPTRWSDGRLRFL
PBANKA_1422900	1	MTNVVCTFKTTPETAKAPDNAIWNRFQYCDEKGWYSLSNHDEITLRPTTFNDGRIKFL
TGGT1_216650	60	DPIDELPEPEKAVQSGKFDVKCWKRGDCKLGIEGDKTVFLKSPISPDVAVYVHAERLPTF
PBANKA_1422900	61	POLDTIPEEFESVLCGKYDAKAWGKDDCNLVIEGDKDVHISLPLGKEKINYNEKERFPTF
TGGT1_216650	120	PKSWKPLVFILNOSLAFRLTENLCLLVVAEKDKTMNISCVDYNGGFACHPSTNMVVAY
PBANKA_1422900	121	LKNSKIIVSLLNENLTVIRINLETALLICINEKKSIVVKSINFNNGFACVNPYSNLAIITY
TGGT1_216650	180	GSYVLKNFEKTPSCQAIPKMLTASGDWGFVQFYFWGFFFPKSVELTRFQAVLGAVGMG
PBANKA_1422900	181	GGFAFN---DLKKCEIVPTITHSCCEWAFFVHLFKWGHIVIPKDLEIKIPSSGL--KLIG
TGGT1_216650	240	KKVDTIGLVFHPNMFINVKLDIPAKTTRALQFGKDFQVTAKRTSETDIEVFLVIDGQLA
PBANKA_1422900	236	KKVDTIAIVSLPPNIIQIHVKIDGPK-CIRKVEYGQDYNITAIKSSSEDIDYVLFQGLL
TGGT1_216650	300	KYNYSFDIRLNKPERPKHTDNIHFKCSCDAEKKKPDPKFKLSACKDSVILLEGQCPSGN
PBANKA_1422900	295	KYEFSDTRLNKEGKGKSIHNAKLKCTSKSKE---VSTFIFQESPNCKVLLGSCNCPDNL
TGGT1_216650	360	PDDQLVSEQLIACFDAEVCLYSTHPPALKLCDAFDVAIRE---
PBANKA_1422900	351	LGH-MLCNQTISIFDAEIGEYQSHPOGLLLTEAFEKLSYPVENA

Appendix Figure S2 | (A) Clustal Omega Multiple sequence alignment of *Plasmodium* spp. (B) Clustal Omega Multiple sequence alignment with the *T. gondii* orthologue. Potential N-terminal palmitoylation site is highlighted in red.

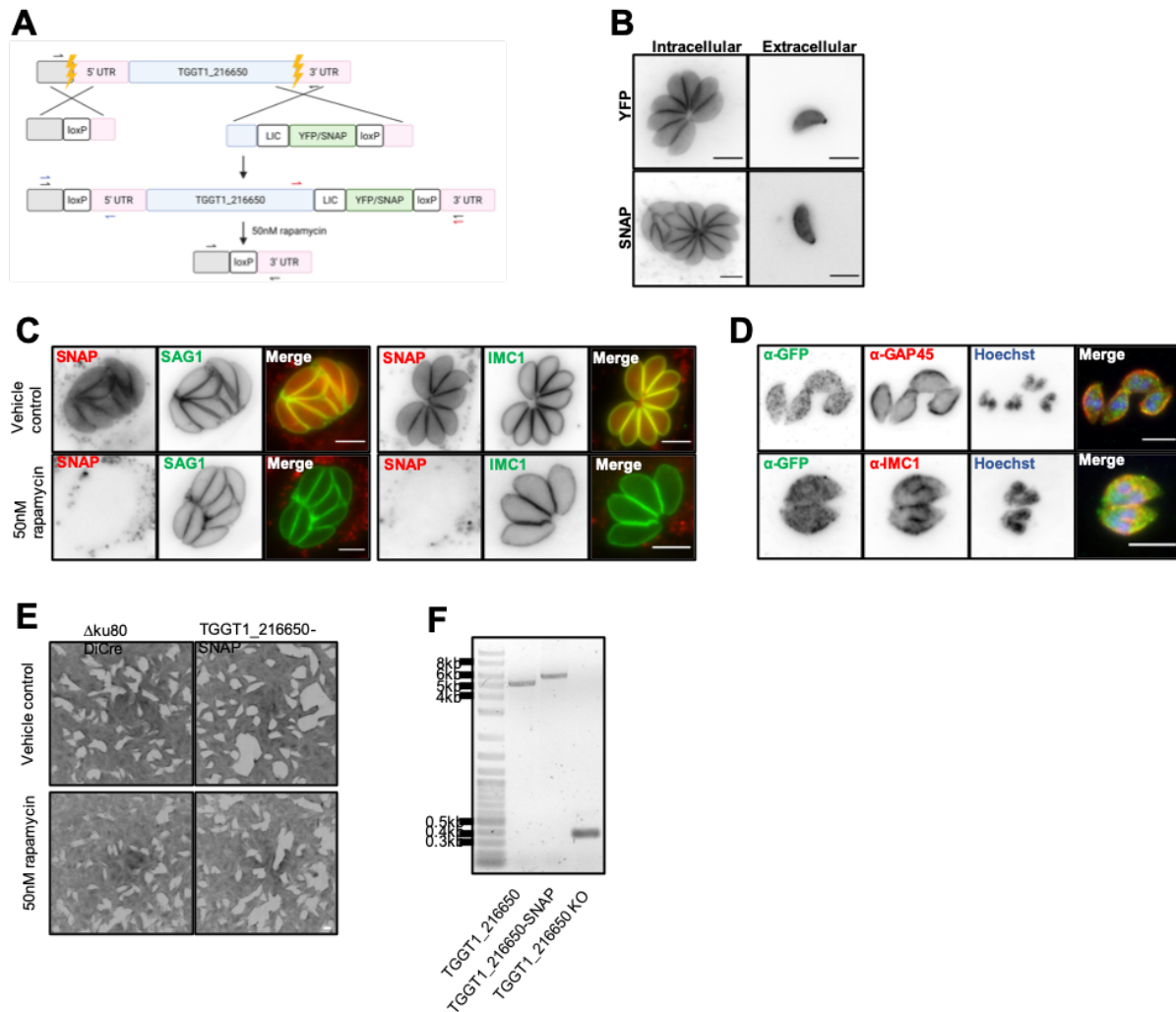
Appendix Figure S3

Genotyping of pL 24 PBANKA_142290 concavin(-) and concavin(-) NS



Appendix Figure S3 | Generation of *concavin(-)* and *concavin(-) NS* parasites via double homologous recombination. (A) Cartoon showing the cloning strategy and primers used for genotyping. (B) Genotyping PCRs of non-clonal *concavin(-)* parasites directly after transfection and after limiting dilution. Agarose gel pictures show 5' integration, 3' integration as well as wildtype and selection marker as indicated in A. Expected amplicon sizes are indicated on the right. (C) Genotyping PCRs of *concavin(-)* parasites after looping out the selection cassette. Expected amplicon sizes are indicated on the right.

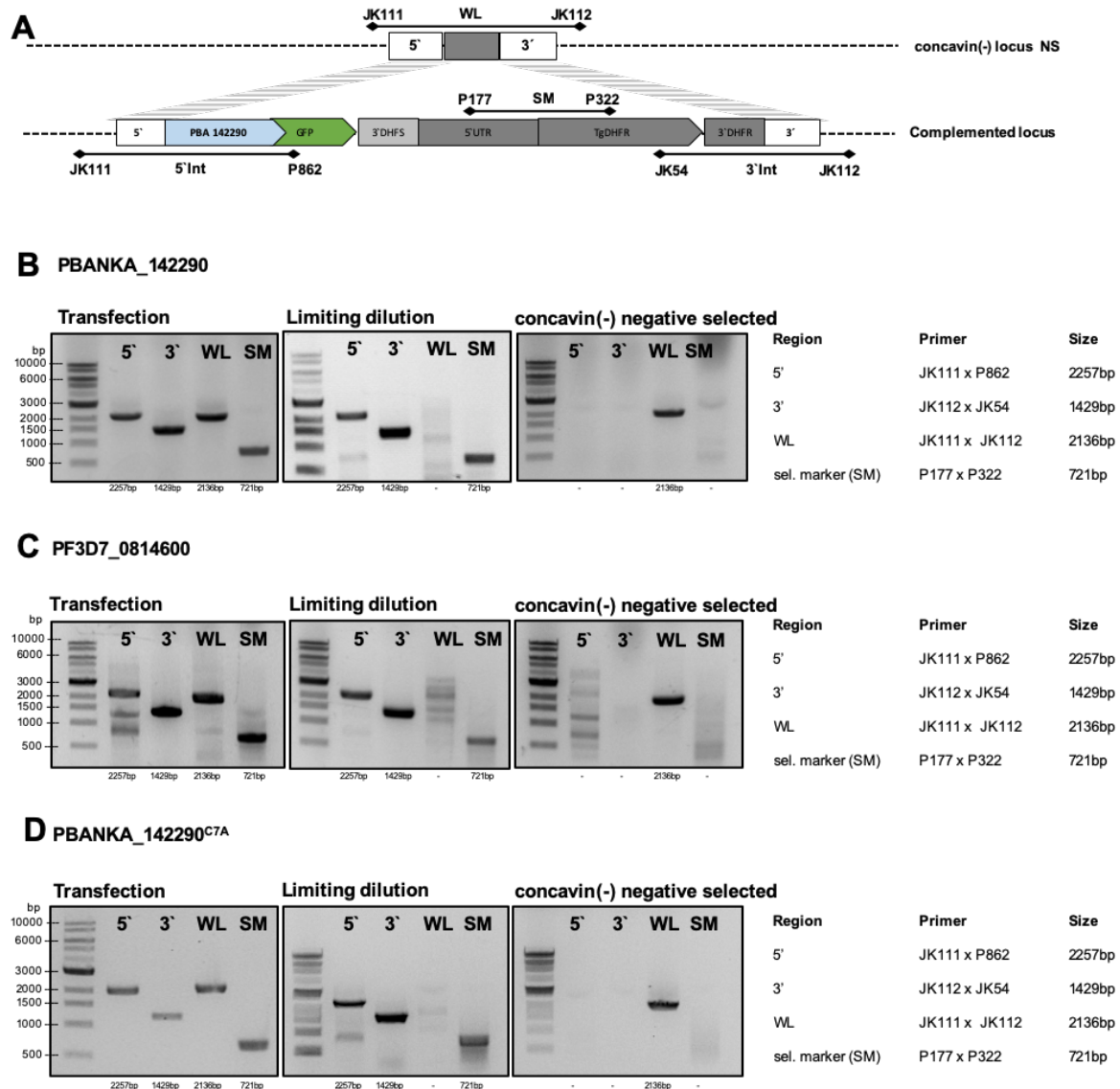
Appendix Figure S4



Appendix Figure S4 | TGGT1_216650 is non-essential in *T. gondii* tachyzoites. (A) CRISPR/Cas9 was used to induce double strand breaks. The DNA repair templates used were designed with homology arms to favour homologous recombination. The approximate position of the 5' UTR was estimated based on the TGME49_216650 annotation on ToxoDB. The LIC sequence was used as a linker between the gene and the tags. Correct integration was confirmed via PCR and sequencing, the primer binding sites as indicated with red and blue arrows. Upon addition of 50 nM rapamycin, the Cre recombinase subunits expressed in the parasite strain dimerise, excising the floxed sequence. (B) Images show the localisation of TGGT1-216650 endogenously tagged with YFP and SNAP tags. Parasites were imaged live in both intracellular and extracellular conditions. (C) SAG1 and IMC1 were internally and C-terminally tagged with HALO-tag respectively using the same protocol as in panel A. Upon knockout of TGGT1_216650, no phenotype was observed. The parasites were imaged live. (D) The parasites were fixed and antibodies were used to amplify the signal. The gene of interest was not observed at the daughter cells while still inside the mother cell during division. (E) 7-day plaque assays. Knockout of the gene of interest following addition of 50 nM rapamycin had no effect on the fitness of the parasites. (F) A knockout line was successfully obtained and can be maintained in culture. Confirmation of successful knockout via both PCR and sequencing, the primer binding sites as indicated with black arrows in panel A. All scale bars: 5 μ m.

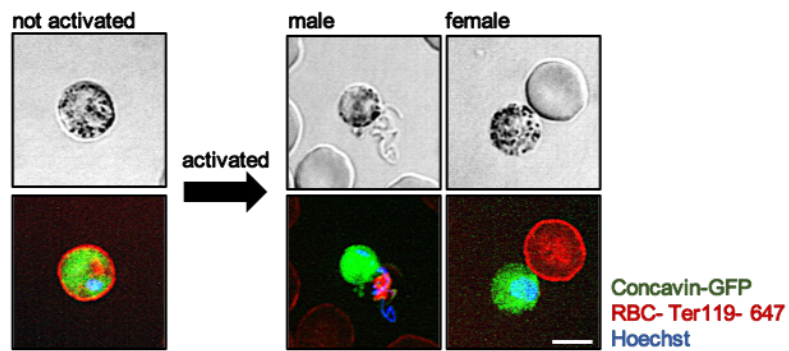
Appendix Figure S5

pL 79 PBANKA_142290-GFP complementation – *Concavin(-)*^{Pb}Concavin
pL 82 PF3D7_0814600-GFP complementation – *Concavin(-)*^{Pf3D7}Concavin
pL 120 PBANKA_142290^{C7A}-GFP complementation



Appendix Figure S5 | Generation of *concavin(-)|P.berghei-gfp*, *concavin(-)|P.falciparum-gfp* and *concavin^{C7A}* parasites via double homologous recombination into *concavin(-)* NS parasites. (A) Cartoon showing the cloning strategy and primers used for genotyping. (B) Genotyping PCRs of the non-clonal *concavin(-)|P.berghei-gfp* parasite line directly after transfection and after limiting dilution. Agarose gel pictures show 5' integration, 3' integration as well as wildtype and selection marker as indicated in A. Expected amplicon sizes are indicated on the right. (C) Genotyping PCRs of the non-clonal *concavin(-)|P.falciparum-gfp* parasite line directly after transfection and after limiting dilution. Agarose gel pictures show 5' integration, 3' integration as well as wildtype and selection marker as indicated in A. Expected amplicon sizes are indicated on the right. (D) Genotyping PCRs of the non-clonal *concavin^{C7A}* parasite line directly after transfection and after limiting dilution. Agarose gel pictures show 5' integration, 3' integration as well as wildtype and selection marker as indicated in A. Expected amplicon sizes are indicated on the right.

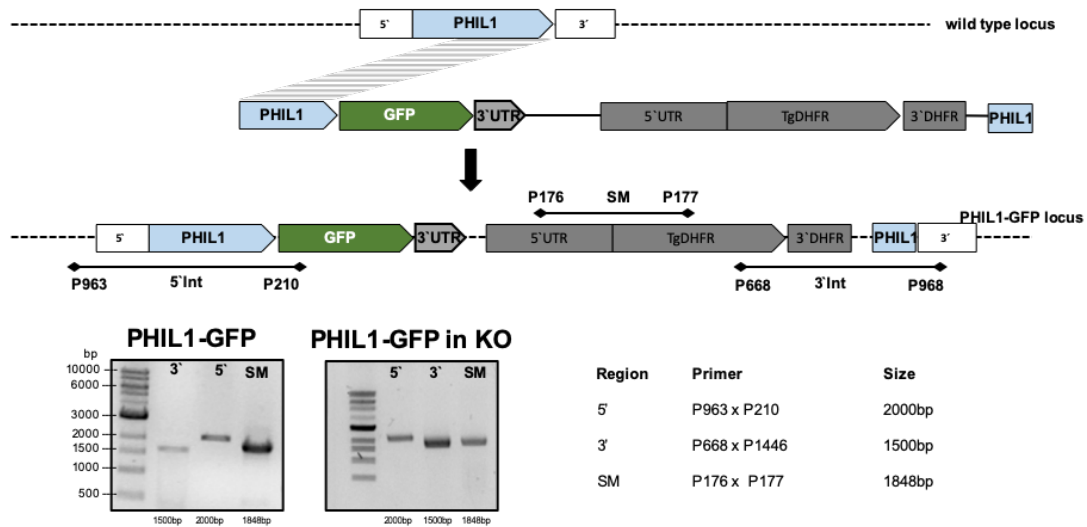
Appendix Figure S6



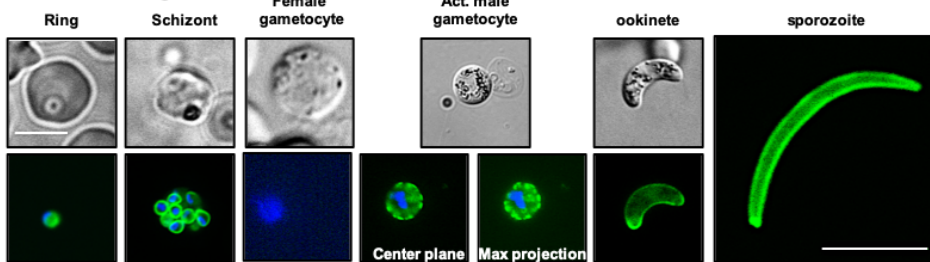
Appendix Figure S6 | Concavin(-)^{Pbconcavin-gfp} localisation in non- activated and activated gametocytes. Red blood cell membrane is stained with an anti Ter- 119 - 647(red) antibody and nuclei (blue) are stained with Hoechst. Scale bar 5 μ m.

Appendix Figure S7

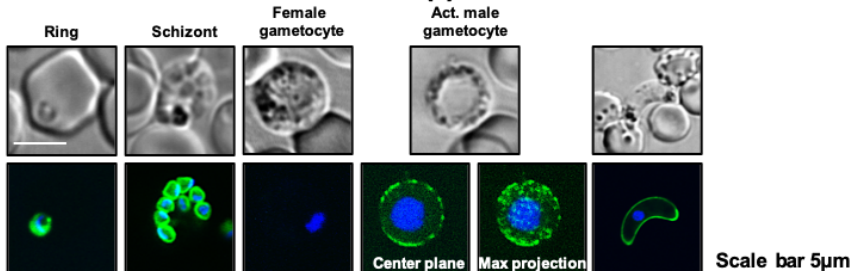
A Generation of PHIL1-GFP and PHIL1-GFP in PBANKA_142290(-) NS



B PHIL1-GFP

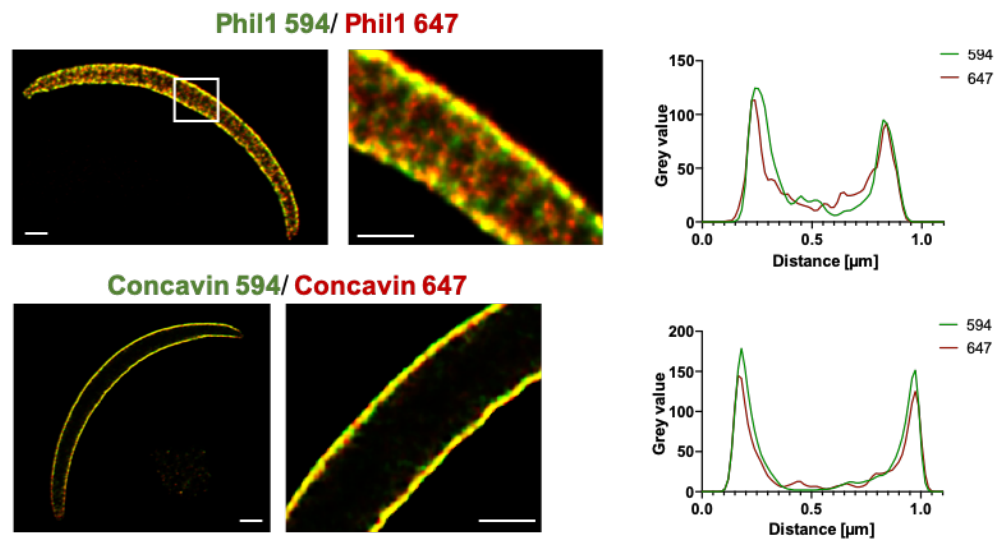


C PHIL1-GFP in concavin(-)



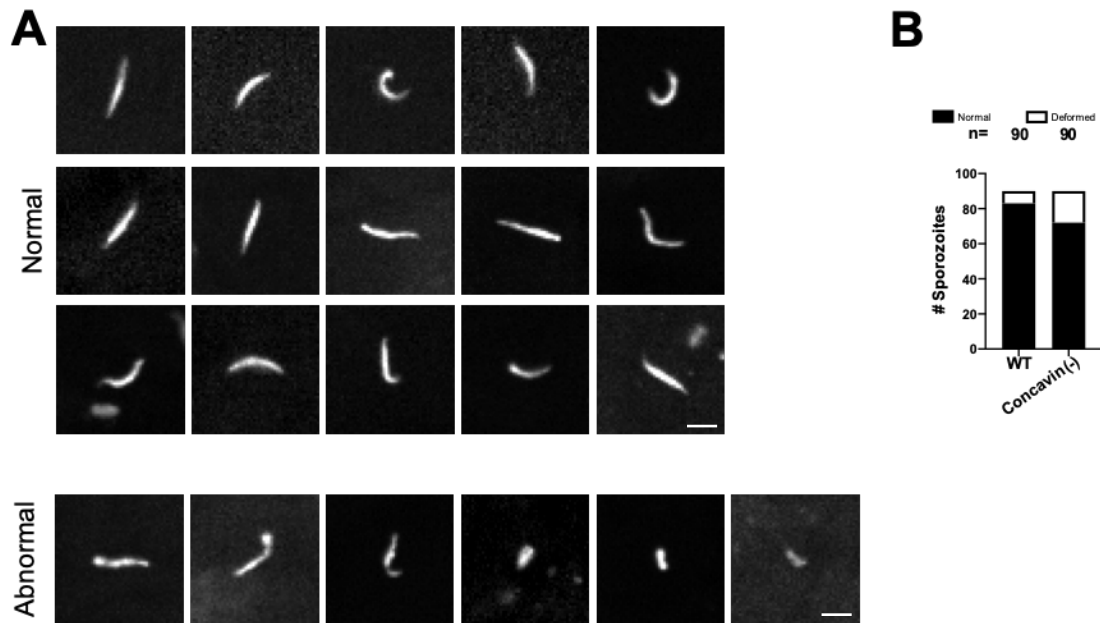
Appendix Figure S7 | (A) Generation of *phil1-gfp* and *concavin(-)|phil1-gfp* parasites via single homologous recombination. The cartoon shows the cloning strategy and primers used for genotyping. Agarose gel pictures shows genotyping of the non-clonal parasites in the wild type as well as in the *concavin(-)* background. (B) Localization of PhiL1-GFP (green) in wild type parasites. Nuclei (blue) are stained with Hoechst. Scale bar 5 μ m. (C) Localization of PhiL1-GFP (green) in *concavin(-)* parasites. Nuclei (blue) are stained with Hoechst. Scale bar 5 μ m.

Appendix Figure S8



Appendix Figure S8 | Control images used for STED. Bleed through of signal in cells stained with atto-594 into the 647 channel resulted in overlays with almost no difference in distance between the 2 channels. Images were deconvolved using the Richardson-Lucy algorithm. The distance between the 2 signals was measured using the plot profile of the respective channels in Fiji (Figure 3C). Measurements and plot profiles taken at the center of the cell. Scale bar 1 μm.

Appendix Figure S9



Appendix Figure S9 | Examples from a representative bite site after transmission of *concavin(-)* parasites. (A) Morphology of normal and abnormal shaped *concavin(-)* sporozoites at the bite site. Scale bar 5 μ m. (B) Normally or abnormally shaped WT or *concavin(-)* sporozoites deposited in the skin. 90 sporozoites were observed for both parasite lines.

Appendix Table S1

Primer sequences used for cloning and sequencing.

Name	Sequence
JK 54	AAAGCGCCGCCGTTTTCTTACTTATATATTTATACCAA
JK 55	AAAGGTACCCGCATATCCTCATATATAATAAATTACCA
JK 56	TTATCATAAAAGCTTGGCTGTCTT
JK 57	AAAGCGCCGCAACAAACAAATCTTCATGTTTGT
JK 58	AAACCGCGGTGATATATGTACTCTTTTGTGTTCC
JK 111	AACACCACTCTGACACCAATTC
JK 112	CCAGATCCAGTATTTTATACCATAGATG
JK 176	GCAGCATTTTCTACTGGATAAGACAG
JK 177	AAATTCGAAATGACAAACATTATAGAATGTACGTTCAAG
JK 178	GGTTCCTTGTTCCAATGGATATGACAAAG
JK 179	AAATTCGAATTTGGAATATAACAAAAAATATATCTCGTAATATA
JK 236	ATGACAAATGTTGTAGAAGCTACTTTTAAACC
JK 237	CTAGGATCCTTAGGCGCCTTTGTATAGTTCATCCATGCCATGTC
P 136	CGCAATTTGTTGTACATAAAATAGGC
P 176	CTAGACAGCCATCTCCATCTGG
P 177	ATGCATAAACCGGTGTGTCTGG
P 210	TTAACATCACCATCTAATTCAACAAG
P 322	CCCCGTTGTCTGAGAAGG
P 587	CTTTGGTGACAGATACTAC
P 668	TGATTAGCATAGTTAAATAAAAAAAGTTG
P 862	TCCAGTGAAAAGTTCTTCTCCT
P 961	ATCCTCTGGTAATTTTTCG
P 963	TAAGCAGTCGACCTACACAATCATGCATACTATGCC
P 969	TAAGCAGAATTCCCCTTCCGAACAAATTTACGCC
P 970	ATGGATCCaccaccaccaccaccaccaccCATATTATCTTTAGGGC
P 1446	CAGCTGCTGGGATTACACATG